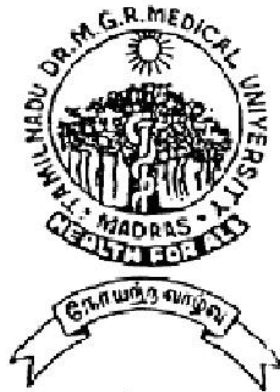


**PREVALENCE OF DERMATOPHYTOSIS
IN A TERTIARY CARE CENTRE**

DISSERTATION SUBMITTED FOR

**BRANCH – IV - M.D. DEGREE
(MICROBIOLOGY)**

MARCH 2009



**THE TAMILNADU
DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI, TAMILNADU**

BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **“PREVALENCE OF DERMATOPHYTOSIS IN A TERTIARY CARE CENTRE”** submitted by **Dr. M. SUDHA** to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D degree Branch11– IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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DECLARATION

I, **Dr. M. SUDHA** declare that, I carried out this work on, **“PREVALENCE OF DERMATOPHYTOSIS IN A TERTIARY CARE CENTRE”** at the Institute of Microbiology, Madurai Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree examination in Microbiology.

Place : Madurai

Dr. M. SUDHA

Date :

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INTRODUCTION

Fungi are widely found in the environment and most of them are harmless commensals, contaminants or non pathogenic agents. There are atleast 100,000 named species of fungi of which less than 500 are associated with human and animal disease.⁴

The incidence of mycotic infections is increasing. A major contributor to the emergence of fungal infections is the increasing number of immunocompromised individuals especially Acquired immuno deficiency syndrome. The fungi are now recognized as significant cause of morbidity and mortality among humans. They have emerged as an important etiological agent of opportunistic infections.⁴

Fungal infections are broadly classified as superficial, subcutaneous, and systemic mycosis. Superficial or cutaneous mycosis are caused by fungi that infect only the superficial keratinized tissue (skin, hair and nails). The most important of these are dermatophytes.²⁸

Skin infections due to dermatophytes have become a significant health problem affecting children, adolescents and

adults. Accurate diagnosis is important to initiate appropriate treatment and also essential for epidemiological purposes. In the background of immunosuppression, detection of these agents becomes mandatory for the effective management of cutaneous mycosis.

The dermatophytes are hyaline septate molds with more than 100 species described. Nearly 40 % of these are associated with human disease. According to Emmon's morphological classification, the dermatophytes are classified into three anamorphic genera -Trichophyton, Microsporum and Epidermophyton based on conidial morphology.¹

Some species of dermatophytes are endemic in certain parts of the world and have a limited geographic distribution. T.soudanense, T.gourvilii and T.yaoundii are restricted to Central and West Africa. M.ferrugineum predominates in Japan. T.concentricum is confined to islands in the South pacific. The increasing mobility of the world's population is disrupting several epidemiological patterns. Some dermatophytes like E.floccosum, T.rubrum and T.tonsurans are globally distributed.⁴ The

dermatophytes manifest as infections of keratinized tissue like skin, hair, nails etc., of humans and animals.

Dermatophyte infections can be treated effectively if timely and proper diagnosis is made. The different syndromes of ringworm infections require different treatment regimens. Generally topical therapies are used for localized or mild infections and oral antifungals for more extensive infections in addition to topical therapy. The newer Azoles such as Fluconazole or Itraconazole and Terbinafine are now the preferred oral drugs to griseofulvin for extensive or severe dermatophytosis. Although Griseofulvin is cheaper it is less effective.

Though various topical and systemic agents are available for the treatment of dermatophytosis, chronic dermatophytosis is commonly seen. Chronic dermatophytosis is a refractory condition which runs a course of one year with remissions and exacerbations.

Resistance to antifungals have been reported with dermatophyte infections especially in nail infections. The resistance pattern can be studied using various methods like macrodilution, microdilution, disc diffusion etc.,

Though various Indian and International studies on epidemiology of dermatophytes are available, no such study has been carried out in Madurai. Since Government Rajaji Hospital, (GRH) Madurai is the largest tertiary care hospital affiliated to Madurai Medical College catering to the needs of lakhs of people from the southern districts of Tamilnadu, the present study was carried out among patients attending GRH and the data were analysed with reference to objectives.

Efforts have been made to compare the prevalence of dermatophytosis in non HIV and in HIV patients. In addition, antifungal susceptibility tests for selected isolates to Fluconazole and Terbinafine was performed.

AIMS AND OBJECTIVES

1. To find out the prevalence of dermatophytosis in HIV infected and noninfected patients.
2. To isolate and identify the common species of dermatophytes and correlate with HIV status.
3. To perform antifungal susceptibility test for the isolates.
4. To correlate with demographic status among various clinical presentations.
5. To compare the present observation with published reports.

REVIEW OF LITERATURE

HISTORY

Because of its visibility, ring worm has been noted and described from the earliest historical times. Growth of the fungus in skin and scalp is almost equal in all directions and the lesions tend to creep in a circular or ring form . So the Greeks named the disease ‘herpes’. The Romans associated the lesions with insects and named the disease ‘tinea’ meaning any small insect larva . This name is retained in the clinical term.¹

Robert remak and Johan L. Schoenlein did extensive studies on scalp and beard mycosis especially favus which is a disfiguring dermatophyte infection of the scalp. In 1834, Remak examined material from favus and noted the presence of filaments resembling molds and concluded that favus was a disease caused by plants². David gruby in 1841 described the isolation of the fungus of favus on potato slices and the production of disease by inoculation of fungus on to normal skin.³

Raymond sabouraud conducted studies on dermatophytes from 1892 to 1936. He published his monumental work ‘Les

Teignes' in 1910 where he classified dermatophytes into four genera, Achorian, Epidermophyton, Microsporum and Trichophyton. In 1934, Chester Emmons modified the taxonomic scheme and classified dermatophytes into three genera excluding achorian based on conidial morphology.⁴

Dawson and Gentles in 1959 discovered the teleomorphic state of Trichophyton ajelloi using 'hair bait' of Van breuseghemii⁵. Griffin and Stockdale in 1960 found out the teleomorphic state of Microsporum gypseum.

Gentles discovered griseofulvin in 1958 which revolutionized the treatment of dermatophytes⁶. In 1980, Azole derivatives and allied group of antifungal drugs were discovered which made a significant impact in the treatment of dermatophytes.

EPIDEMIOLOGY

Venugopal et al⁷ in an article on Antimycotic susceptibility testing of dermatophytes, have quoted that dermatophytes are the major agents of cutaneous mycosis and remain a general health problem.

Attapattu et al⁸ in, 'A study of tinea capitis in Srilanka' has said that the study of dermatophytosis in a population is

important as it may reflect the climatic condition , customs , hygiene and socio economic status of people .

Karauri and Seim⁹, on “Incidence of dermatophytosis in Kuwait” states that ring worm infections are more common in very low income groups and less common in moderately rich groups .

Ranganathan et al 1995¹⁰ On Effect of socio economic status on the prevalence of dermatophytosis in Madras, has said that prevalence of dermatophytosis in low socio economic status may be due to poor personal hygiene and illiteracy. Environmental conditions like hot temperature and humid weather increases incidence of dermatophytosis.

Venkatraman et al¹¹ in his article has stated that dermatophytosis is the most common skin disease in the rural population in and around Chennai, Tamil nadu, India .

Rao ¹², in his article on ‘Mycotic diseases in India’ says that India is a large sub continent with remarkably varied topography, situated with in tropical and sub tropical belt of the world. It’s climate is conducive to the acquisition and maintenance of mycotic infections. Classifying dermatophytes by their usual

habitat is useful for understanding their various clinical presentation and patterns of transmission.

Geophilic organisms grow in the soil and only sporadically infect humans. They cause inflammatory lesions in humans. Strains of *Microsporum gypseum*, the most common geophilic pathogen when cultured from humans are more virulent than those cultured from soil.¹³

Zoophilic species are usually found on animals and sporadically transmitted to humans. Domestic animals and pets are becoming an increasing source of these infections in urban areas e.g *Microsporum canis* in cats and dogs. Transmission may occur through direct contact with the animal or indirectly by fomites. Although human infections with zoophiles is often suppurative, animal infection may be silent showing the unique adaptation of the fungi to animal host.¹⁴

The Newzealand dermatological society has stated in an article that dermatophyte spores can live for more than a year in human scales in the environment.

The dermatophytes exclusively affecting humans are called anthropophilic. They cause relatively mild and chronic

infections in humans, produce few conidia in culture and may be difficult to eradicate.

Host variability also affects clinical presentation. Immuno compromised individuals are more susceptible to severe or refractory dermatophytosis and advances in chemotherapy and transplant medicine has led to an increase in opportunistic infections by previously non pathogenic dermatophytes.¹⁵

Interestingly only the severity of dermatophytosis is increased with HIV disease and not the prevalence.¹⁶

Age, sex and race are additional important epidemiological factors, as shown by the fact that dermatophytosis is more prevalent in males than females.¹⁷

Prasad, Priya et al¹⁸ on “A study of chronic dermatophytosis in a rural hospital in Chidambaram” has pointed out that, the common age group of dermatophytosis is 20 -30 years, males are more commonly affected than females and tinea corporis is the commonest infection followed by tinea cruris.

Human travel may influence the distribution of epidemic fungi e.g *Trichophyton tonsurans* has replaced *Microsporum audouinii* as the predominant cause of tinea capitis in the USA, correlating well with the immigration of Mexican and caribbean population.¹

Local customs influence the prevalence of dermatophytosis e.g use of macerating occlusive foot wear has made tinea pedis and onychomycosis much more common in industrialized nations.¹

Genetics play a role in dermatophytosis. In households afflicted with dermatophytosis relatives are more likely to be infected than conjugal partners even with equal exposure to the fungus.¹⁹

CLINICAL PRESENTATIONS

Dermatophyte infections are one of the earliest known fungal infections of mankind and are very common through out the world. Although dermatophytosis does not cause mortality, it does cause morbidity and poses a major health problem²⁰ especially in tropical countries like India due to the hot and humid climate. No race in any geographical location is totally free of dermatophytosis.¹

Kanwar et al ²¹ in IADVL text book of dermatology has stated that tinea corporis is the commonest clinical type of dermatophyte in India followed by tinea cruris.

Kaur from Chandigarh, Vasu from Warangal and Malik , Chugh, Prakash^{22,23,24} in their separate studies have concluded that tinea capitis is less common in India than in other countries .

Hajini et al ²⁵ on “Effect of hair oils on the growth of dermatophytes” and Garg et al²⁶ on “Inhibition of growth of dermatophytosis by Indian hair oils” infer that the use of hair oils in India customarily have been shown to have an inhibitory effect on dermatophytosis in vitro.

TINEA CAPITIS is a dermatophytosis of the scalp and associated hair. The most common cause world wide is *Microsporum canis*. It is commonly found in children aged 3 to 14 years. Two types are seen –the ectothrix type and the endothrix type. In ectothrix, arthrospores are seen outside the hair shaft. *M.audouinii*, *M.canis*, *T.verrucosum* and *T.mentagrophytes* cause these infections. In endothrix, arthrospores completely fill the hairshaft. *T.tonsurans*, *T.violaceum* causes this²⁷. Favic type of scalp infection is caused by *T.schoenleinii*.²⁸

Phil pt ²⁹ on some aspects on the epidemiology of tinea, has commented that tinea capitis is universally reported as a disease of children. The post pubertal changes in hormones results in acidic sebaceous gland secretions which are responsible for decrease in incidence with age.

TINEA CORPORIS is ringworm of the glabrous skin except the palm, sole and groin . T.rubrum, T.mentagrophytes and E.floccosum commonly causes tinea corporis. Tinea corporis resulting from T.rubrum is often extensive ³⁰. For any part of the world the cause of tinea corporis can be assessed by reference to the prevailing dermatophyte flora in the region.³¹

TINEA BARBAE (sycosis barbae) is a dermatophyte infection of the facial terminal hair of men. Dermatophytosis of the same area in females and prepubertal males involve glabrous skin and is termed tinea faciale. Mostly it is caused by the zoophilic organisms T.mentagrophytes and T.verrucosum and rarely M.canis.¹

TINEA CRURIS (dhobie itch) is ring worm of groin. T.rubrum , T.mentagrophytes and E.floccosum causes this. It is prevalent in tropical countries because warm, humid condition is

important for this infection³². It is common in men than in women.³³

TINEA UNGUIUM is dermatophyte invasion of nail plate. Principal species involved are *T.rubrum*, *T.mentagrophytes*, *E.floccosum* and *T.violaceum* . Ring worm of nails occur in all parts of the world and almost all dermatophytes have been reported to infect nails at one time or other³⁴

In an article on ‘onychomycosis in Hongkong’³⁵, it is reported that dermatophytosis is the commonest cause of onychomycosis.

DIAGNOSIS

The diagnosis of dermatophytosis is based on a combination of clinical observation supplemented by laboratory investigation. The history of the patient is essential regarding the age, occupation, hobbies, living conditions, onset, duration and course of disease as well as intake of previous treatment.⁴

Clinically the distribution, type of lesion, concurrent disease and constitutional symptoms of the patient should be seen. In the laboratory, diagnosis depends on the demonstration of

causative pathogens in tissue by microscopy and isolation of fungus in culture.

Direct microscopic examination of material from the lesion is not a sensitive test for detecting dermatophytosis, but it is the most rapid method of determining the etiology of an infection when the test is positive³⁶

Suman and Beena on the profile of dermatophyte infection in Baroda and Kannan. Janaki, Chennai^{37,38}, have inferred that KOH positivity is seen in about 70 % of cases and culture positivity is seen in about 45 % of samples.

Fouzan, Nanda, kubek³⁹, have stated that the possible reason for negative culture from microscopically positive sample may be that highly contaminated samples were grown over by fast growing saprophytic species which prevented the growth of dermatophytes even on a medium with cycloheximide.

Peerapur , Inamdar et al on⁴⁰ ‘clinico mycological study of dermatophytosis in Bijapur’, has reported that *Trichophyton rubrum* is the commonest organism isolated followed by *Trichophyton mentagrophytes*.

Fitz Patrick et al⁴¹ has stated that *Trichopyton violaceum* is the most frequent invader of the scalp.

ANTIFUNGAL SUSCEPTIBILITY TESTING :

With increasing incidence of resistance, the need for antifungal susceptibility testing is gaining importance. As with antibacterial compounds, tests designed to find the MIC are said to be the most dependable means of determining the relative effectiveness of different antifungal compounds and of detecting the development of drug resistant organisms⁴⁴

The CLSI M27 A document has given guide lines for susceptibility testing of filamentous fungi. Using the format of M27A, a microdilution type of antifungal susceptibility testing for dermatophytes is universally accepted. Some studies show that invitro susceptibility test by macrobroth dilution also gives reliable and definite results⁴⁵

Venugopal pankajalakshmi, venugopal tarakalakshmi⁴⁸, in their article on 'AST testing for 85 isolates of dermatopytes with itraconazole and ketoconazole 'have stated that MIC of itraconazole was lower than ketoconazole .

They have also studied the antifungal activity of 7 antifungal drugs, Ketoconazole, Miconazole, Itraconazole, Naftifin, terbinafine and griseofulvin by agar dilution technique and found Terbinafine the most effective with a MIC₅₀ of 0.01 micrograms/ml. The MIC₅₀ of Econazole, Naftifine and Itraconazole was 0.1 microgram / ml. The MIC₅₀ for Ketoconazole , Miconazole and Griseofulvin was 1microgram / ml

TREATMENT

The choice of proper treatment is determined by the site and extent of infection and the species involved, as well as the efficacy , safety profile and kinetics of the available drugs.⁴²

For localized non extensive lesions, topical therapies are generally used. For tinea unguium, tinea capitis and extensive tinea corporis, systemic antifungal treatment is necessary.²⁸

Griseofulvin, the oldest antifungal agent for dermatophytosis is now being replaced by Azoles and allylamine groups because of the broader spectrum of activity and better tolerance.

Azoles include Miconazole, clotrimazole, ketoconazole, econazole Fluconazole, Itraconazole and voriconazole. The azoles affect the cell membrane synthesis through inhibition of cytochrome p-40 dependent 14 alpha methylation. They are all fungistatic.

Allylamines are Terbinafine and Naftifine. They inhibit Squalene epoxidase thereby suppressing ergosterol biosynthesis and causing toxic accumulation of squalene within fungal cell wall. Terbinafine has a very high level of invitro activity against dermatophytes. It is fungicidal⁴³

Khalid Abdel kabeer from King Saud university⁴⁶, has stated that Terbinafine is the most effective drug in the treatment of dermatophytosis followed by Fluconazole, Itraconazole and griseofulvin . Both MIC and MFC of terbinafine is lower than other drugs . Terbinafine was also found to be the most effective antifungal agent in invitro study .

Samia Girgis, et al⁴⁷ have pointed out that terbinafine is the most powerful anti fungal agent .

MATERIALS AND METHODS

The materials [Fungal scrapings] were obtained from patients, attending the Dermatology outpatient department and Anti Retroviral Therapy Centre (ART) of Govt. Rajaji Hospital, Madurai during a period of three months ie. from February to April 2007. Ethical committee clearance was obtained prior to the onset of the study and informed consent was obtained from each participant.

INCLUSION CRITERIA

1. Suspected dermatophyte lesions of the skin, hair and nails of HIV infected and uninfected patients were considered for the study irrespective of a) age, b) sex, and c) socioeconomic status

EXCLUSION CRITERIA

1. All other superficial mycosis like Tinea versicolor, piedra were excluded from the study.
2. Patients with history of diabetes, malignancy and patients taking immuno suppressive therapy were excluded from the study.

For the clinically diagnosed dermatophytosis, case details regarding age, sex, occupation, educational status, socioeconomic status, personal hygiene were recorded. Type of lesion and the area of distribution of lesion were noted. (copy of proforma enclosed).

One hundred and thirty suspected dermatophytosis cases were selected from 1000 immunocompetent persons. Scraping was done for these cases. HIV testing was carried out for the 130 individuals.

Simultaneously, 25 suspected dermatophytosis from 200 confirmed HIV positive cases attending ART centre, GRH, Madurai were selected. Scraping was done for these cases. CD4 count was performed for these 25 patients.

SPECIMEN COLLECTION :

The specimens collected were skin scrapings, hair clippings along with scalp scrapings, and nail clippings .

The affected areas were cleaned with 70% alcohol and specimen of skin, hair, nail were taken with the help of sterile scalpel and scissors as the case may be. Scraping was done after wearing protective gloves.

In the case of skin lesions, with the help of sterile scalpel, scrapings were taken from the margin of the lesions as the centre of the lesion usually heals quicker and the margin had the proliferative and growing fungal elements.

In the case of hair, the affected hair shafts were taken including the roots and scrapings were taken from the surrounding scalp area.

Nail clippings were taken using a nail cutter and the subungual area was scraped using a blunt scalpel. Care was taken to obtain nail material from the advancing infected edge closest to the cuticle, where the likelihood of viable hyphae is the greatest.

The specimens were collected in individual sterile folded squares of paper that permitted drying of the specimen, reduced the bacterial contamination and also provided conditions under which specimens may be stored for long periods without appreciable loss in the viability of ringworm fungi. Processing of specimens was done on the same day of the collection of specimen.

DIRECT MICROSCOPIC EXAMINATION

The samples were studied directly in 10% potassium hydroxide and examined under the microscope for evidence of branching septate hyphae, arthrospores .

KOH preparation was made by emulsifying the specimen in a drop of 10% KOH on a clean microscopic slide. The KOH specimen mixture was allowed to stand for 10 minutes before examination in the case of skin and about 30 minutes in the case of nails and hair at room temperature.

A coverslip was applied and the specimen was examined for the presence of narrow regular hyphae that characteristically break up in to arthroconidia. In hairs, endothrix and ectothrix types of infection were observed.

After examining the KOH mounts, the remaining specimen was used for culture. All specimens were cultured irrespective of the direct KOH mount result.

CULTURE

The standard media for primary isolation of dermatophyte namely Sabouraud's dextrose agar, containing chloramphenicol (0.04gms/litre) and cycloheximide (0.5g/litre) was used. The

specimens were inoculated on to two sets of Sabouraud's dextrose agar and in each more than four implants were made for increasing the chances of isolation. One inoculated slant was kept at room temperature and the other was incubated at 37⁰C. The cultures were maintained for 30 days before discarding them as negative.

Growth was relatively slow, usually seven days to three weeks were required. When growth became evident on the primary isolation media, fungi were identified macroscopically on the basis of colony appearance, pigmentation, consistency [granular, gritty or velvety nature of the colony] and microscopically by the appearance of conidia – both micro and macro, that were variable from one species to another.

For observing the microscopic appearance, using teasing needle, mounts from the culture were made in Lactophenol cotton blue [LCB]. With a pair of dissecting needles, a small portion of the colony was taken and placed on a microscopic slide in a drop of LCB. Then the colony was teased apart with needle. A cover slip was placed over the specimen and gentle pressure was applied on the surface of the coverslip to disperse the mount. The preparation was then examined under 10 x and 40 x objectives.

RIDDLE'S SLIDE CULTURE METHOD

A sterile petridish of 135 mm size was taken and was labeled with the specimen number and date of inoculation. A grease free sterile glass slide was placed in the above and from the already prepared Sabouraud's agar, a square block of agar was cut and placed on the glass slide and from the primary inoculation of the growth of the concerned fungus, the sides of the agar block was inoculated. This was covered with sterile cover glass . 20% glycerol was kept in a sterile screw cap of the universal container inside the petridish in order to keep the atmosphere humid. This was incubated at 26°C for one to two weeks by which time adequate growth of the fungus was obtained.

The slide culture allowed observation of the fungus while it was growing . When spores were evident, a lactophenol cotton blue mounting was made on the coverslip after gently lifting it with a sterile forceps . Then the agar block was removed and the slide was stained with LCB which served as the second mount.

The primary fungal growth was further subcultured on Potato dextrose agar for bringing out better pigmentation and to improve

conidiation. The urease agar media was used to differentiate between *T.rubrum* and *T.mentagrophyte* species.

CULTURAL CHARACTERS OF ISOLATED FUNGI :

TRICHOPHYTON RUBRUM :

Growth on SDA took about a week. The colony surface was initially white and consistency was cottony or granular. The reverse of the colony showed red pigment that diffused into the agar.

Microscopically the microconidia were tear shaped, distributed on either side of the hyphal strands producing a 'birds on a fence' appearance . Multicellular, elongated, pencil shaped macroconidia with smooth thin walls characteristic of *T.rubrum* were seen .

TRICHOPHYTON MENTAGRAPHYTES :–

Growth on SDA was seen in a week. Colony surface was initially white and became cream coloured on maturity. The reverse of the colony showed characteristic tan colour and some showed red colour similar to *T.rubrum* .

Microscopically, the microconidia were arranged in loose grape like clusters. Macroconidia were long, multicelled and pencil

shaped with smooth thin walls. Characteristic spiral hyphae were seen in some.

Trichophyton rubrum and *mentagrophytes* were differentiated using urease test . A piece of colony was inoculated in Christensen's urease agar and a positive urease test was observed in case of *T.mentagrophytes* within 4 days .

TRICHOPHYTON VIOLACEUM

Growth was slow and took three weeks to appear. At first, colonies were cream coloured and later developed a purple colour and became folded. The reverse pigment was also purple in colour.

Microscopically chains of intercalary and terminal chlamydoconidia and swollen hyphal cells were seen. Macro and microconidia were not seen.

MICROSPORUM GYPSEUM :

Growth was observed within 5 days. The colonies were initially white turning brown on maturing. The macroconidia were numerous, elliptical shaped with echinulate walls. Microconidia were very few.

EPIDERMOPHYTON FLOCCOSUM

The colonies were observed within 6 days. Initially, they were grey white and developed a khaki green pigment when mature. The centre of the colony was folded. The reverse pigment was yellow brown. Club shaped macroconidia were numerous arranged singly and in clusters. Microconidia were absent .

ANTIFUNGAL SUSCEPTIBILITY TESTING

T.rubrum and *T.mentagrophytes* were selected for antifungal susceptibility testing by microdilution method as they were the predominant species isolated. Also, both these species showed good conidiation when subcultured in potato dextrose agar which is essential for anti fungal susceptibility testing. Antifungal susceptibility test was carried out for 13 isolates of *T.rubrum* and 4 isolates of *t.mentagrophytes* from both HIV and non HIV patients.

INOCULUM PREPARATION :

The selected dermatophyte isolates were inoculated on potato dextrose agar slant and incubated at 28⁰C for one week until good conidial growth occurred. Then the colonies were covered with approximately 10 ml of distilled water and a suspension was prepared by gently probing the colonies with a sterile swab. The

resulting suspension was transferred to a sterile tube. Heavy particles were allowed to settle for 5 – 20 minutes and the upper homogenous suspension was collected and vortexed. The density of the suspension was adjusted to 0.5 McFarland standard. Then the suspension was mixed with RPMI 1640 medium.

MEDIUM

RPMI 1640 broth with L-Glutamine and without sodium bicarbonate was used with phenol red as indicator. The medium was buffered to a PH of 7 with 0.165M morpholine propane sulfonic acid.

ANTIFUNGAL AGENTS

Distributed IV formulation of Fluconazole at a concentration of 2000 µg per ml and Pure powder of Terbinafine were used.

For Terbinafine , the pure powder was weighed to obtain a concentration of 1000 µg /ml . This was done by using the formula

$$\text{Weight(mg)} = \frac{\text{Volume (ml)} \times \text{Concentration (}\mu\text{g/ml)}}{\text{Potency (}\mu\text{g/mg)}}$$

Then 5ml of polyethylene glycol 400 [PEG] was taken in a glass tube. The weighed terbinafine powder was added, vortexed

and dipped in a boiling water bath. This was repeated till the solution became clear. This solution was the stock solution. 1ml of stock solution was mixed with 4ml PEG400 to get a concentration of 200 µg/ml. This was again diluted 1:10 by adding 0.4ml of the above solution with 3.6 ml PEG-400. Then serial dilutions of 0.4ml of the above solution was done using PEG so that the concentration of the drug in the plates was from 0.04 µg to 10µg.

For Fluconazole, the IV formulation with a concentration of 2000µg/ml was the stock solution. 1.3 ml of stock solution was added to 2.7 ml sterile distilled water to get a concentration of 640 µg /ml. This was further serially diluted to achieve a concentration of 0.125µg/ml to 64 µg.

PROCEDURE :

Microdilution plates with 96 ‘U’ shaped wells were used. Rows two to twelve contained the series of drug dilution in 100 µl volume and row one contained 100 µl of drug free media which served as growth control .

With the addition of 100 µl of inoculum to the micnotitre wells containing the drug dilutions, the concentration of terbinafine

was from 0.002 μ g to 1 μ g. The concentration of fluconazole was from 0.06 μ g to 32 μ g.

The microplates were incubated at 37⁰C and was read at 3, 7, and 14 days of incubation. The Minimum Inhibitory Concentration (MIC) was determined by visual inspection of the growth inhibition of each well compared to that of the growth control well. The MIC ie. the lowest concentration of the antifungal agent that causes a specified reduction (80% or more) in growth was found out.

RESULTS

A total of 1000 patients attending skin OPD were first screened for the presence of dermatophytosis and 130 cases were included for the study. Simultaneously 200 HIV patients, attending ART unit at GRH were screened for dermatophytosis and 25 patients were included for the study. The distribution of the cases is given in Table no.1.

Table -1

Screened cases	Total cases	Dermatophytosis	%
Non HIV	1000	130	13
HIV Positive	200	25	12.5

It was found that the prevalence of dermatophytosis clinically was independent of HIV status.

All the 130 non HIV cases and 25 HIV positives with dermatophytosis were further analysed age wise, and it was found that among the 130 non HIV cases analysed, 5 were in the age group of 0-10 (3.84%), 11 in the age group 11-20 (8.46%), 21 in the age group 21-30 (16.15%) 53 in the age group 31-40 (40.76%), 28 in the age group of 41-50 (21.5%), and 12 in the age group 51-60 (9.23%).

Among 25 HIV positive cases with dermatophytosis, 11 were in the age group 21 to 30 [44%] and 14 were in the age group 41-50 [56%]. This is given in Table number 2.

Table – 2
AGE WISE DISTRIBUTION

AGE IN YEARS	Cases in Non HIV	%	Cases in HIV	%
0 - 10	5	3.84	-	-
11 – 20	11	8.46	-	-
21 - 30	21	16.15	11	44
31 – 40	53	40.76	14	56
41 - 50	28	21.54	-	-
51 - 60	12	9.23	-	-

It was observed that the highest number of dermatophytosis was seen in the age group of 31-40 years in both non HIV and HIV cases.

130 samples from non HIV cases were analysed, sex wise and it was found that 81 were males (62.3%) and 49 were females (37.7%).

Out of 25 HIV cases, 18 (72%) were males and 7 (28 %) were females.

The above findings are shown in table no. 3.

Table : 3

Sex wise distribution in Non HIV and HIV individuals

Sex	No.cases in Non HIV	%	No.of cases in HIV	%
Male	81	62.3	18	72
Female	49	37.7	7	28

In both conditions, males were more commonly affected

All the 130 non HIV and 25 HIV positive samples were analysed as per the socio economic status mainly involving the income, educational status and occupation.

TABLE – 4
DERMATOPHYTOSIS AND SOCIO-ECONOMIC STATUS

	No.cases in Non HIV	No.cases in HIV
Group – I (Very low) Income < 1000 / month Occupation - daily wages labourer Education - Illiterate / primary school	71 (54.6%)	9 (36%)
Group - II (Low) Income < 2000 / month Occupation – peon / Driver etc Education - High school	44 (33.8%)	16 (64%)
Group – III (Middle) Income > 2000 / month Occupation – Clerk / Nurses etc Education - Diploma / Graduates	15 (11.5%)	-

The above table shows that more of non HIV cases are in group I while HIV cases are seen more in group II.

For the 25 HIV cases with dermatophytosis, CD4 counts were analysed. 5 cases (20%) were with CD4 count 0 - 99 and 6 cases (24%) were with CD4 100-199 and CD4 200-299 each and 4 cases (16%) were with CD4 count 300-399 and 400 to 499 each.

Table – 5

CD4 count in PLHA cases with dermatophytosis

CD4 count	No.of cases	Percentage
0 – 99	5	20
100 – 199	6	24
200 – 299	6	24
300 – 399	4	16
400 – 499	4	16

Occurrence of dermatophytosis does not depend on levels of CD4 count in the individual.

The samples were further analysed depending upon the clinical manifestations and it was found that 74 cases out of 130 had tinea corporis (56.9%), 37 out of 130 had tinea cruris (28.5%), 7 had tinea faciei (5.4%), 5 had tinea capitis (5.4%) and 7 had tinea unguium (5.4%).

In HIV cases, 60% had tinea corporis while 36% had tinea cruris. One case [4%] had tinea faciei.

TABLE – 6
CLINICAL PRESENTATION

DIAGNOSIS	Non HIV	%	HIV +	%
Tinea corporis	74	56.9	15	60
Tinea cruris	37	28.5	9	36
Tinea faciei	7	5.4	1	4
Tinea capitis	5	3.8	-	-
Tinea unguium	7	5.4	-	-
Total	130	100	25	100

It was observed that the commonest clinical lesion in this study was tinea corporis (56.9%) followed by tinea cruris(28.5%) in both non HIV and HIV + patients.

In the age wise correlation with clinical presentation, in the case of immunocompetent, 5 tinea capitis were seen in the age group 0-10 yrs. Between 11-20 years, 3 tinea faciei and 8 tinea corporis cases were seen. Between 21-30 years, 1 tinea faciei, 10 tinea corporis, 7 tinea cruris and 3 tinea unguium were seen. 2 tinea faciei, 30 tinea corporis, 18 tinea cruris and 3 tinea unguium were seen between 31-40 years. 1 tinea faciei, 19 tinea corporis, 7 tinea cruris and 1 tinea unguium were seen between 41-50 years. 7 tinea corporis and 5 tinea cruris were seen between 51-60 years.

In HIV patients, 7 tinea corporis, 1 tinea faciei and 3 tinea cruris were seen in the age group 21 – 30. Between 31 -40 yrs. 8 tinea corporis and 6 tinea cruris cases were seen.

Table – 7
AGE AND CLINICAL PRESENTATION

Age yrs	T. Capitis		T.Facei		T.Corporis		T.Cruris		T.Unguium	
	N	HIV	N	HIV	N	HIV	N	HIV	N	HIV
0- 10	5 3.8%	-	-	-	-	-	-	-	-	-
11 – 20	-	-	3 2.3%	-	8 6.2%	-	-	-	-	-
21 – 30	-	-	1 0.8%	1 4%	10 7.7%	7 28%	7 5.4%	3 12%	3 2.3%	-
31 - 40	-	-	2 1.5%	-	30 23.1%	8 32%	18 13.8%	6 24%	3 2.3%	-
41 - 50	-	-	1 0.8%	-	19 14.6%	-	7 5.4%	-	1 0.8%	-
51 - 60	-	-	-	-	7 5.4%	-	5 3.8%	-	-	-
Total	5	-	7	1	74	15	37	9	7	-
N = non HIV										

It was observed that tinea corporis was the predominant clinical presentation in both HIV and non HIV, the common age group being 21-40 years.

Tinea capitis was the only clinical presentation of dermatophytosis seen in the age group 0-10 years.

In sex wise correlation of clinical presentation in Non HIV persons, among males 43 had tinea corporis, 27 had tinea cruris, 5 had tinea faciei, 2 had tinea capitis and 4 had tinea unguium. So in males, tinea corporis was the commonest lesion followed by tinea cruris.

Among female, 31 had tinea corporis, 10 had tinea cruris, 2 had tinea faciei, 3 had tinea capitis and 3 had tinea unguium. Here also tinea corporis was the commonest lesion followed by tinea cruris

In HIV patients, tinea corporis was seen in 9 males [36%] and 6 females [24%]. Tinea cruris cases were seen in 8 males [32%] and 1 female (4%). Tinea faciei was seen in one male (4%).

TABLE – 8 SEX AND CLINICAL PRESENTATIONS

DIAGNOSIS	MALE				FEMALE			
	N	%	HIV	%	N	%	HIV	%
Tinea corporis	43	33.07	9	36	31	23.8	6	24
Tinea cruris	27	20.8	8	32	10	7.7	1	4
Tinea faciei	5	3.8	1	4	2	1.5	-	-
Tinea capitis	2	1.5	-	-	3	2.3	-	-
Tinea unguium	4	3.07	-	-	3	2.3	-	-
N= non HIV								

It was observed that the tinea corporis was predominant in both males and females in HIV and non HIV cases.

In non HIV, out of 130 samples, 112 were positive by KOH mount (86%) and 100 showed culture positivity (77%). In HIV, out of 25 samples, 21 were positive by KOH (84%) and 17 were positive by culture (68%).

TABLE –9
DIRECT KOH MOUNT AND CULTURE POSITIVITY

Diagnostic Methods	No.of Samples (Non HIV)			No. of Samples (HIV)		
	Collected	Positive	%	Collected	Positive	%
KOH Mount Positive	130	112	86.15	25	21	84
Culture Positive	130	100	76.92	25	17	68

KOH positivity was more than culture positivity in HIV and non HIV cases.

On analysing the 100 dermatophyte species isolates from Non HIV cases, 74 cultures were *T.rubrum* (56.92%), 22 isolates were *T. mentagrophytes* (16.92%), 2 isolates were *T.Violaceum* (1.54%), one *E.floccosum* and one *M.gypseum*.

Of the 17 species isolated from HIV cases, 13 cases (76%) were *T.rubrum* and 4 (24%) were *T.mentagrophytes*.

TABLE –10 : DERMATOPHYTE SPECIES ISOLATED

SPECIES	Non HIV	%	HIV	%
T. rubrum	74	74	13	76
<i>T. mentagrophytes</i>	22	22	4	24
<i>T. violaceum</i>	2	2	-	-
<i>E. floccosum</i>	1	1	-	-
<i>M. gypseum</i>	1	1	-	-
Total	100	100	17	100

***T.rubrum* was the most prevalent species isolated followed by *T.mentagrophytes* in non HIV and HIV cases.**

On analyzing the species involved in various dermatophytes in both non HIV and HIV cases, it was seen that in non HIV cases *T.rubrum* was isolated from 47 tinea corporis cases,(63.5%), 22 tinea cruris cases (59.5%), 1 from tinea faciei and 4 from tinea unguium. *T.mentagrophytes* was isolated from 14 tinea corporis cases, 3 tinea cruris, 1 tinea capitis and 4 tinea faciei. *T. violaceum* was isolated from 2 tinea capitis cases. *E. floccosum* was isolated from one tinea cruris case and *M.gypseum* from 1 tinea faciei case.

TABLE – 11
SPECIES INVOLVED IN VARIOUS DERMATOPHYTOSIS (N)

Species	T.corporis	T.cruis	T.capitis	T.faciei	T.unguium
T.rubrum	47 (63.5%)	22 (59.5%)	–	1 (14%)	4 (57%)
T.mentagrophytes	14 (18.9%)	3 (8.1%)	1 (20%)	4 (57%)	–
T.violaceum	–	-	2 (40%)	–	–
E.floccosum	–	1 (2.7%)	–	–	-
M.gypseum	–	–	–	1 (14%)	–

From the above table, it is seen that tinea corporis and tinea cruris are predominantly caused by *T.rubrum* in non HIV cases. All tinea unguium cases are caused by *T.rubrum*. *T.violaceum* was involved in T.capitis and *E.floccosum* in T.cruis only.

Of the 17 isolates from HIV patients, *T. rubrum* was isolated from 9 (60%) tinea corporis cases and 4 (44%) of tinea cruris cases. *T. mentagrophytes* was isolated from 2 (22%) of tinea cruris and 1 (7%) of tinea corporis cases.

Table - 12
SPECIES INVOLVED IN VARIOUS DERMATOPHYTOSIS (HIV)

Species	T.corporis	T.cruris	T.capitis	T.facei	T.unguium
<i>T. rubrum</i>	9 (60%)	4 (44%)	-	-	-
<i>T. mentagrophytes</i>	1(7 %)	2(22%)	-	1	-
<i>T. violaceum</i>	-	-	-	-	-
<i>E. floccosum</i>	-	-	-	-	-
<i>M. gypseum</i>	-	-	-	-	-

The above table shows that tinea corporis and tinea cruris were predominantly caused by *T. rubrum* in HIV patients also.

In antifungal susceptibility testing, Terbinafine was put up in dilution from 0.002 µg to 1 µg. In this study the MIC of T.rubrum and T.mentagrophytes against terbinafine in both HIV and non HIV was in the 0.002 to 0.03 µg range. Fluconazole was put up in dilutions from 0.06 µg to 32 µg. The MIC of T.rubrum and T.mentagrophytes against Fluconazole was in the 0.5 to 4 µg range.

Table – 13 ANTIFUNGAL SUSCEPTIBILITY TESTING

Fluconazole MIC Sensitivity Range (0.5 to 4 µg)										
	Non HIV					HIV				
Species	No.	S	%	R	%	No.	S	%	R	%
T. rubrum	13	11	85	2	15	13	13	100	-	-
T. mentagrophytes	4	4	100	-	-	4	4	100	-	-
S = Sensitivity, R = Resistant										

Table – 14

Terbinafine MIC Sensitivity Range (0.002 to 0.03 µg)										
	Non HIV					HIV				
Species	No.	S	%	R	%	No.	S	%	R	%
T. rubrum	13	13	100	-	-	13	13	100	-	-
T. mentagrophytes	4	4	100	-	-	4	4	100	-	-
S = Sensitivity, R = Resistant										

It was seen that all isolates of T.rubrum and T.mentagrophytes were sensitive for Terbinafine and the MIC range of Terbinafine is less than fluconazole.

DISCUSSION

India is a large subcontinent with remarkably varied topography, situated within the tropical and subtropical belts of the world. Its climate is conducive to the acquisition and maintenance of mycotic infections. It is accepted worldwide that dermatophytes grow well in hot humid conditions. In two studies done in Korea^{50,51} it has been found that dermatophyte prevalence is high during summer months. Considering the above facts this study was conducted during the summer months February 2007 to April 2007.

Impaired cell mediated immunity is one among the various factors that play a role in acquisition of dermatophytosis. Rook's text book of dermatology says, there is a strong evidence that the development of cellular immunity via sensitized T lymphocytes is a key factor in immunological defence against dermatophytosis. HIV is one disease which shows definite defective cell mediated immunity. So in this study, the prevalence of dermatophytosis in non HIV and HIV patients were compared.

The initial screening of cases in the present study did not show significant variation in the prevalence of dermatophytosis between HIV and non HIV individuals irrespective of the CD4 count. Similar study by Rodwell et al⁶¹ and Burkhardt et al⁶² also revealed that there is no

relationship between HIV infection or reduced CD4 count and the prevalence of dermatophyte infection. In this study, though the prevalence was the same clinically in HIV and non HIV cases, severe and invasive lesions were seen in HIV cases while the lesions were restricted to stratum corneum in non HIV cases. This correlates with Burkhardt et al⁶² study where it is given that dermatophytosis in immunocompromised host is more severe than in immunocompetent host. In non HIV cases, non specific host defence mechanism like activation of serum inhibiting factors and complement prevents invasiveness of dermatophytes. In HIV, this immune mechanism is impaired which leads to invasiveness of dermatophytes.

In this study, it was observed that 40.76% non HIV and 56.25% HIV cases were affected with Dermatophytosis in the age group 31-40 years. Similar study by Prasad P.V.S. et al¹⁸ also showed that the common age group involved in Dermatophytosis is 21-40 yrs. Kaviarasan et al⁵⁹ in his study also reported that the mean age of dermatophytosis in HIV cases was 30.7 years. The present observation correlate with previous publications. It is obvious that the mean age of 30 years is the period where the labourers exert more physically, resulting in increased perspiration which produces a hot, humid, environment in the body, favoring the growth of Dermatophytes.

Excessive perspiration also washes away fungus killing oils in the skin making it more prone to dermatophyte infection.

In the present study, non HIV males [62.3%] were more affected than non HIV females [37.7%]. The male:female ratio is 1.8 : 1. This correlates with other studies by Prasad PVS et al ¹⁸ Suman et al ³⁷ and SS Sen et al ⁵² where the male : female ratio was 1.75 : 1.1. Peerapur BVet al ⁵³ and Philpot CM ¹⁷ have observed that higher incidence in males might be due to greater physical activity and increased sweating. In the present study, the male cases were mostly labourers and coolies working in sunlight most of the time leading to profuse sweating which in turn resulted in increased dermatophyte infection. Similarly in HIV patients also, males with dermatophytosis (72%) were more than females with dermatophytosis (28%). The male female ratio was 2.5 : 1. Kaviarasan et al and Rajesh et al ⁶⁰ also revealed that males were more affected than females in HIV patients. In addition to the environmental factors in HIV, immunosuppression was another added factor for the predominance of dermatophytosis in males.

In this study, it was found that 54.6% dermatophytosis in non HIV were in socio economical group I and 33.8% were in socio economical group II Ranganathan et al ¹⁰ and Karauri et al¹¹ in similar studies also pointed out that dermatophytosis was more common in the

group I due to poor hygiene and illiteracy. Studies by Pandhya AA et al⁶³ and Das gupta et al⁶⁴ stated that changing and washing undergarments were practiced very rarely in group 1 and group 2 which accounted for the increased incidence of dermatophytosis in these groups. In the present study, Group 1 people live in overcrowded areas. Infrequent washing of clothes, poor maintenance of personal hygiene and sharing things commonly might have paved the way for increased incidence of dermatophytosis in this group. In this study, 64% of HIV patients with dermatophytosis were in group II and 36% in group I. Similar study by Kaviarasan et al⁵⁹ also showed that majority of HIV patients with dermatophytosis in his study were mostly drivers. The group II in this study also involved drivers, skilled labourers and peons who attended ART clinic in GRH regularly. Long distance drivers represent one of the risk groups for acquisition of HIV. Ultimately, drivers with HIV infection and added physical exertion might have resulted in increased incidence of dermatophytosis in this group.

Of the 130 non HIV cases analysed in this study, tinea corporis was the commonest presentation[58.8%] followed by tinea cruris [12.3%] which corresponds to Kanwar AJ et al study⁵⁴ Prasad PVS et al¹⁸, Suman et al³⁷ who have also showed that tinea corporis was present in 52.8% cases and tinea cruris in 15.6% cases. In HIV patients also, it was

shown in this study that *T.corporis* was present in 60% and *T.cruis* in 36%. Rippon's text book of mycology states that complete anaerobiosis due to tight clothing, maceration and high rate of sweating in waist, groin and other hairy regions makes these sites more vulnerable to dermatophytosis. Constant sweating keeps the temperature in these regions at 27°C which is a favourable condition for the growth of dermatophytes. All these factors might have contributed to the increased incidence of tinea corporis.

In this study, tinea capitis was seen in 3.8% of non HIV cases and no tinea capitis was seen in HIV cases. All tinea capitis cases were seen in the age group of 0 - 10 years. This corresponds to the study by Philpot²⁹, in which he reported that tinea capitis was a disease of children. He proved that post pubertal changes in hormones resulted in acidic sebaceous gland secretions which were responsible for decrease in incidence with age. In the present study there were no HIV positive children in the age group 0-10 years and so no *T.capitis* cases were seen in HIV cases. Kaur et al²², Vasu et al²³, Malik et al²⁴ in their studies have concluded that tinea capitis is less common in India than in other countries. Hajini et al²⁵ and Garg et al²⁶ have shown that the customary use of hair oils (particularly mustard oil) in India have been shown to have an inhibitory effect on dermatophytes invitro.

In this study, the diagnosis of dermatophytosis in non HIV and HIV cases were made by demonstrating dermatophytes under microscope by KOH mount and culturing the specimen on SDA with cycloheximide media and proved that direct KOH mount was found to be a good screening test for dermatophytosis because 86.2% of non HIV and 84% of HIV samples were positive in KOH mount while 76.9% of non HIV and 68% of HIV cases only were positive in culture. The study by Kannan, C.Janaki et al³⁸ and Suman singh et al³⁷ also showed that KOH mount positivity was seen in 80% of cases. But in contrast to this study, the culture positivity was only 45%.

The isolation of fungus by culture depends on the quality of media, the pH maintenance, time of collection and processing of specimens and certain other environmental factors. In the present study, the media used was Hi Media's Cycloheximide agar and processing was done on the same day itself, which contributed to the increased isolation by culture. Culture positivity also depends on the viability of organism, sufficiency of sample, site of sample collection and proper processing of samples. As all these were followed in this study the isolation rate was high.

In the present study, the commonest species isolated from both HIV and non HIV cases was *T.rubrum* [74%] followed by

T.mentagrophytes [22%]. Mary Elizabeth rushing⁴⁰ in her study stated that T.rubrum was the commonest species isolated from dermatophytosis. She explained that because of the presence of mannan in the cell wall of T.rubrum, which inhibits CMI, hinders proliferation of keratinocytes and enhances its resistance to skin's innate immunity, T.rubrum resists eradication. Fitz Patrick et al⁴¹ in his study has explained that T.rubrum is an anthropophilic fungus (man loving) adapted to humans as hosts. Anthropophilic infections are transmitted via direct contact or fomites. They are usually non inflammatory which results in a clinically silent carrier state that delays diagnosis and propagate the infection. All these factors might be responsible for the same finding in this study also.

The Antifungal susceptibility testing of fluconazole and Terbinafine revealed that fluconazole was effective with a MIC range of 0.5 to 4 µg and terbinafine was effective with MIC range of 0.002 to 0.03 µg. All T.rubrum and T.mentagrophytes isolates from HIV and non HIV samples were sensitive to terbinafine. Two T.rubrum isolates from non HIV sample were resistant to fluconazole. So in this study Terbinafine was found to be more sensitive than fluconazole. This is in accordance with the study by Pankajalakshmi V et al ⁴⁸ where Terbinafine was the most sensitive drug with MIC₅₀ of 0.01µg/ml.

Similarly a study by Samia A Girgis et al ⁴⁷ also finds Terbinafine to be the most powerful antifungal agent. This is because terbinafine is a proven fungicidal drug while fluconazole is a fungistatic drug. Terbinafine inhibits squalene epoxidase thereby suppressing ergosterol biosynthesis and causes toxic accumulation of squalene in fungal cell wall. Fluconazole inhibits cytochrome dependent 14 alpha methylation and affects cell membrane synthesis. Because of the toxin accumulation, the fungus is killed by Terbinafine, whereas fluconazole inhibits the multiplication of the fungus by affecting the cell membrane synthesis. Hence Terbinafine can be considered as a more effective drug than fluconazole in the treatment of dermatophytosis.

SUMMARY

Dermatophytosis is one of the commonest conditions in patients attending Dermatology out patient department. In view of the immuno compromised status due to disease or drugs, it is expected that the occurrence of dermatophytosis may be more. Keeping this in view the present study was undertaken to find out the prevalence and pattern of dermatophytosis among HIV positive and negative individuals and correlate with selected demographic variables and compare with published reports. Efforts were made to isolate the fungi by standard methods and to perform sensitivity tests.

The prevalence rate of dermatophytosis is the same in both HIV (12.5%) and non HIV cases(13%). 41% of dermatophytosis in non HIV cases and 56% in HIV cases were seen in 31-40 years age group and 60% of the affected were males. 55% of non HIV individual were in socio economic group I and 64% of HIV patients were in socio economic group II. None of the HIV cases showed correlation with CD4 count. 57% of non HIV and 60% of HIV cases presented with tinea corporis as clinical manifestation in the age group 30-40 years affecting both sexes equally. 85% of specimen were positive by KOH

mount and 69% by culture method. 74% of *T.rubrum* was isolated in non HIV cases and 76% in HIV patients and the common clinical lesions by *T.rubrum* was tinea corporis. Terbinafine which is a fungicidal drug was more sensitive than flucanazole by invitro susceptibility test.

CONCLUSION

The following conclusions were arrived

- Clinically the prevalence of dermatophytosis among HIV and non HIV cases was 13% and 12.5% respectively.
- The mean age involved in HIV and non HIV cases was 30 years.
- Dermatophytosis was observed more in males in both HIV and non HIV cases.
- Non HIV individual in the socio economic group I and HIV patients in socioeconomic group II were commonly affected with dermatophytosis.
- CD4 count in HIV has no correlation with dermatophytosis.
- The commonest clinical manifestations of dermatophytosis in both HIV and non HIV cases was tinea corporis irrespective of age and sex.
- The conventional KOH mount was found to be an adequate screening test for dermatophytes and isolation of dermatophytes by proper culture methodology still holds good as a gold standard for confirmation.

- T.rubrum was the commonest species of dermatophyte isolated from both HIV and non HIV cases which presented as tinea corporis in both conditions.
- Invitro susceptibility test for terbinafine and fluconazole on dermatophytes revealed that terbinafine was more sensitive against T.rubrum and T.mentagrophytes than fluconazole.

Suggestions :

1. The reason for lack of increased occurrence of dermatophytosis among HIV cases of this region need further exploration.
2. Since KOH mount is simple and adequate to screen dermatophytosis, medical and paramedical personal may be trained in the same in resource limited settings for practical purposes.
3. Health Education to the patients regarding personal hygiene should be implemented to prevent dermatophytosis.

BIBLIOGRAPHY

1. Rippon JW. 'The pathogenic fungus and the pathogenic Actinomycetes' 3rd edition, Philadelphia, WB Saunders co.
2. Schoenlein J.L 1839, Med zeitung 9;73-74, Arch : Anat, phy, wiss. Med. 82-cited from 'The Dermatophytes' by Irene Weitzman et al, Clinical Microbiology reviews, April 1995.
3. Gruby D 1841 CR Acad Sci [paris] 13:309-312 – cited from Rippon's Text book of Medical Mycology, 3rd edition Philadelphia, WB Saunders Co.
4. Jegadish chander's, 'Text book of Medical Mycology', 2nd Edition-2002, Mehta publishers.
5. Dawson C.O and J.C Gentles 1959 , Nature – London 183:1345-1346 – cited from 'The Dermatophytes' by Irene Weitzman et al, Clinical Microbiology Review, April 1995.
6. Gentles J.C. 1958; Nature London .182:476-477- cited from Rippon's Text Book of Medical Microbiology, 3rd edition Philadelphia, WB Saunders Co.

7. Venugopal PV, Venugopal TV. Antimycotic susceptibility testing of Dermatophytes. Indian Journal of Medical Microbiology 1993;11:151-4.
8. Attapattu MC et al. A study of tinea capitis in Srilanka, Mycology. 1982;27-32.
9. Karauri R, Seim M. Incidence of dermatophytosis in Kuwait. Sabouraudia 1979; 17: 131-7.
10. Ranganathan et al. Effect of socioeconomic status on the prevalence of dermatophytosis in Madras. Indian Journal of Dermatology and Venereology [IJDVL], 1995; 61:1, 16-18.
11. G.Venkatraman et al. African Journal of Microbiology research. 2007 ; 009-012.
12. Rao A. Mycotic diseases in India, a critical review. Bulletin of School of Tropical Medicine, 1959; 13:22.
13. Green DL. Journal of American Academy of Dermatology, 1948;31:S 112.
14. Aly R et al. Journal of American Academy of Dermatology. 1994; 31:S 21.
15. Rinaldi MG. Journal of American Academy of Dermatology. 2000; 43:S 120.

16. Johnson RA. Journal of American Academy of Dermatology. 2000; 43:S 135.
17. Ogawa H et al . Medical Mycology. 1998;36:S 166.
18. Prasad PVS, Priya K et al .Study of Chronic Dermatophytosis in a Rural Hospital in Chidambaram. Indian Journal of Dermatology and Venereology, 2005; 71 : 2.
19. Jones HE et al. Journal of American Academy of Dermatology. 2001; 34:S 145.
20. Emmons CW, Binford CH-Medical Mycology, 3rd edition, Philadelphia, Lea and Febiger 1977;117-67.
21. Kanwar et al, IADVL Text book and Atlas of Dermatology, 2nd edition, Mumbai 2001, 21-58.
22. Kaur S, Incidence of Dermatophytosis in Chandigarh and surrounding areas, Indian Journal of Dermatology and Venereology ,1970;36:143-5.
23. Vasu DRBH. Incidence of Dermatophytosis in Warangal, AP, India. Indian journal of Medicine, 1966;54:468-74.
24. Malik AK, Chugh TD, Prakash K. Dermatophytosis in North India, Indian journal of Pathology and Microbiology.1978 ; 21:53-9.

25. Hajini GH et al. on Effect of hair oils and fatty acid on the growth of Dermatophytosis and their invitro penetration of scalp hair. Sabouraudia, 1970; 8:174.
26. Garg AP et al. Inhibition of growth of Dermatophyosis by Indian hair oils. Mycosis, 1992;35:363-9.
27. Kligman AM. Journal of investigative Dermatology. 1952; 18: 231-40.
28. Rook's text book of Dermatology. 7th edition, 31.28, Blackwell Science Ltd.
29. Phil pt CM. Some aspects on the epidemiology of tinea. Mycopathologia ,1991 ; 3 : 62.
30. Rosman N. British Journal of Dermatology.1996; 78:208-12.
31. Devroey . Semin dermatology, 1985; 4 : 185-200.
32. Amer M Taha M et al. International Journal of Dermatology. 1981; 20: 431-4.
33. Black F Mann SJ et al . Sabouraudia. 1974; 12: 352-61.
34. English MP. Nails and fungi. British Journal of Dermatology,1976; 74: 697-701.
35. Onychomycosis in Hongkong . International Journal of Dermatology , 1997; 36:757-761.

36. Fran fisher, Norma B cook. Fundamentals of diagnostic Mycology, WB Saunders, An imprint of Elsevier Science.
37. Suman.S. and Beena.M. Profile of dermatophyte infection in Baroda. Indian Journal of Dermatology and Venereology, 2003; 69:4, 281-283.
38. Kannan.M. and C.Janaki. Isolation of Dermatophytes. Indian Journal of Medical Microbiology, 2006; 24 :212-5.
39. Fouzan A, Nanda A, Kubek K , Internacional Journal of Dermatology, 1993 ; 32 : 798-801.
40. Mary Elizabeth Rushing - e' Medicine.
41. Fitz Patrick TB et al . Dermatology in General Medicine , 6th edition, McGraw- Hill Medical Publishing Division.
42. Belky's Fernandez, Fransisco Tcabanes. Coloborative evaluation of optimal fungal susceptibility testing conditions of Dermatophytes. British Journal of Clinical Microbiology, 1986 ; 23 : 298-301.
43. Katsambas Lotti. European hand book of Dermatology treatment, 2nd edition.
44. Ashok rattan. Antimicrobials in Laboratory medicine, 1st edition, Churchill Livingstone Pvt Ltd.

45. Lancy J sumangalabai M. Invitro susceptibility testing of clinical isolates of dermatophytes. Journal of Academy of Clinical Microbiologists, 2003; 5,82.
46. Khalid Abdel kabeer M, King Saud University. Survey of human pathogenic Dermatophytes in Riyadh, Saudi Arabia and testing of antifungal agents against these fungi. International Journal of Dermatology, 2001 ; 62 : 58-61.
47. Samia A Girgis, Nehal M et al. Egyptian Dermatology. Online Journal.
48. Venugopal V pankajalakshmi, Venugopal V Tarakalakshmi. Anti susceptibility testing for 85 isolates of dermatophytes with itraconazole and ketoconazole. Indian Journal Of Dermatology and Venereology , 1992 ;58 : 368-371.
49. Pankajalakshmi V.Venugopal , TarakalakshmiV.Venugopal et al.Indian Journal of Dermatology and Venereology ,1993; 62.
50. A Mycological study on Dermatophytosis in Seoul, Korean Journal of Dermatology 1994 ; 32 : 24-33.
51. Clinical and mycological observation of Dermatophytosis in Kangwon Province, Korea, Korean Medical database.

52. SS Sen and ES Rasul, Guwahati Medical College, IJMM 2006 ; 24 : I, 77-78.
53. Peerapur BV, Inamdar AC et al, IJMM, 2004 ; 22 : 4, 273-274.
54. Kanwar AJ, Mamta, Chander J. 'Superficial fungal infections' Valia RG, Valia.
55. P. Veer et al. Study on onychomycosis : prevailing fungi and pattern of infection IJMM 2007 ; 25 : 53-6.
56. Dermatophytosis in Karaj, IJD 2006 ; 51 : 4, 262-264.
57. Dermatophytosis in NW Algeria, Middle East Journal of Scientific Research, 2007 ; 2 (3-4) : 104 – 106.
58. R. Kaur, BKashyap, PBhalla. Onychomycosis IJMM, 2008; 26(2) : 108-16.
59. Kaviarasan PK, Jaisankar TJ, Thappa DM, IJDVL, 2002; 68 : 213 – 216.
60. Rajesh R, Subramanian K et al, Indian Journal of STD 2006 ; 27 (2) : 70-74.
61. Rodwell, Graham EJ et al, International Journal of Dermatology, 2008 ; 47 : 4.

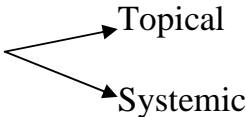
62. Burkhart et al, International Journal of Dermatology, 2003 ;
92 : 10
63. Pandhya AA, Thirumalachar M, 'Dermatophytosis in Poona',
Mycopath mycol Appl, 1970 ; 40 : 225-36.
64. Das Gupta SN, Shoma SK. Mycopath Mycol, appl. 1959; 10 :
177-80.

PROFORMA

Date :

1. Name :
2. Age
3. Sex :
4. Occupation :
5. Socio economic status
6. Address :
7. Educational Status

7. Description of Lesion

- Type of Lesion
- Site of Lesion
- Duration of Lesion
- No.of Lesions
- Treatment taken 
 - Topical
 - Systemic
- Clinical Diagnosis

8. Associating Factors

- Diabetes
- Steroid Treatment & other immuno suppressants
- H/o Trauma
- Contact with Animals
- Cancers

9. Skin scraping on
10. KOH Mount
11. Culture in SDA Agar :
 - Macroscopic :
 - Microscopic (LPCB) :
12. Sub Culture

TINEA CORPORIS



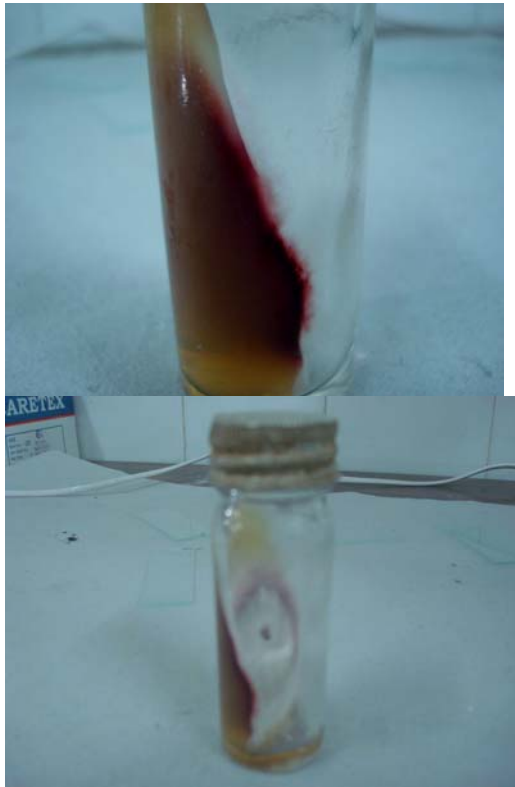
TINEA CAPITIS



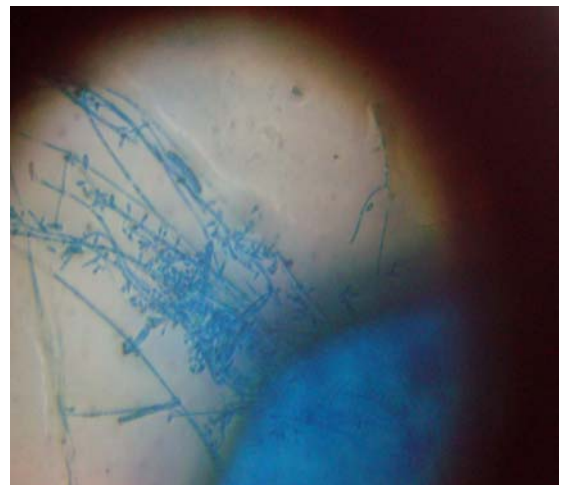
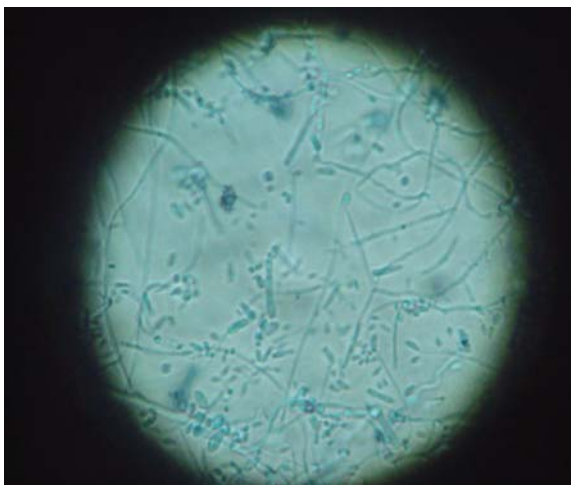
DIRECT KOH MOUNT



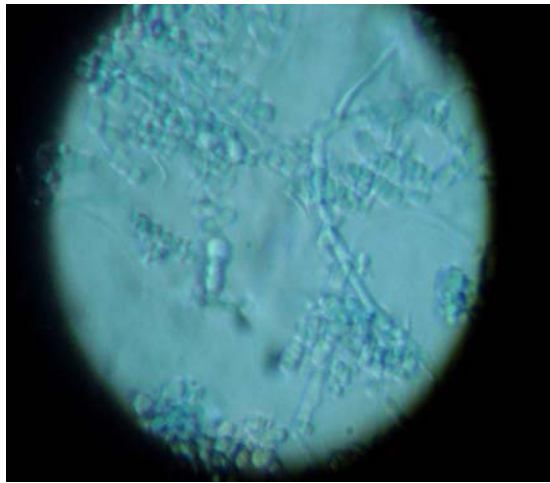
**TRICHOPHYTON RUBRUM
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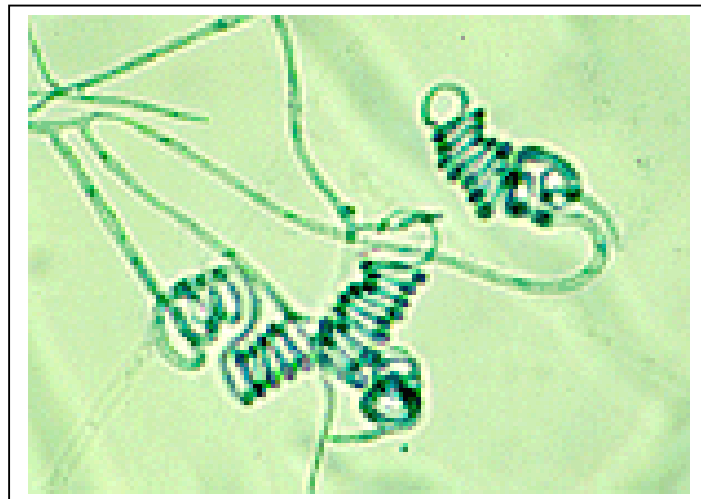
**TRICHOPHYTON RUBRUM
(MICROSCOPIC)**



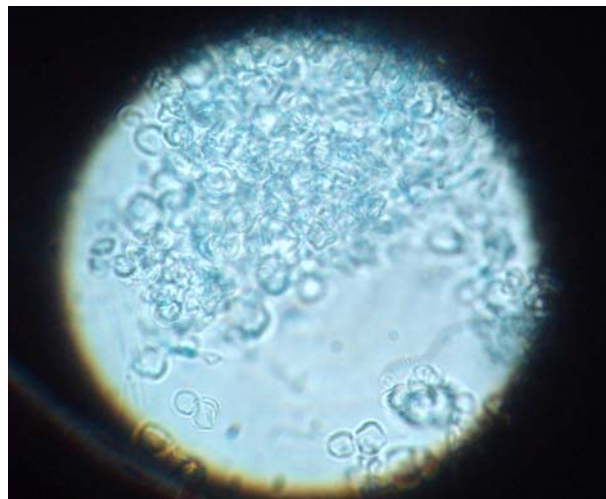
TRICHOPHYTON MENTAGROPHYTES



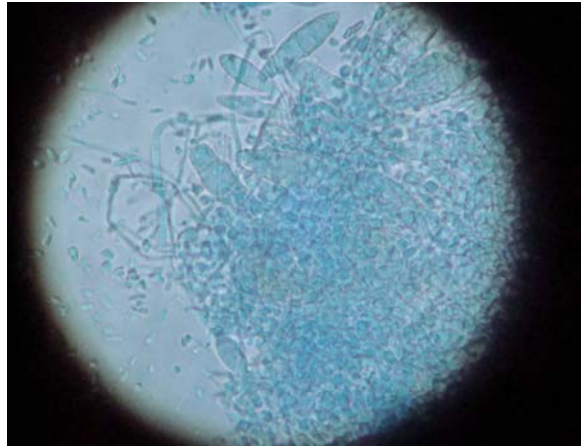
SPIRAL HYPHAE



TRICHOPHYTON VIOLACEUM



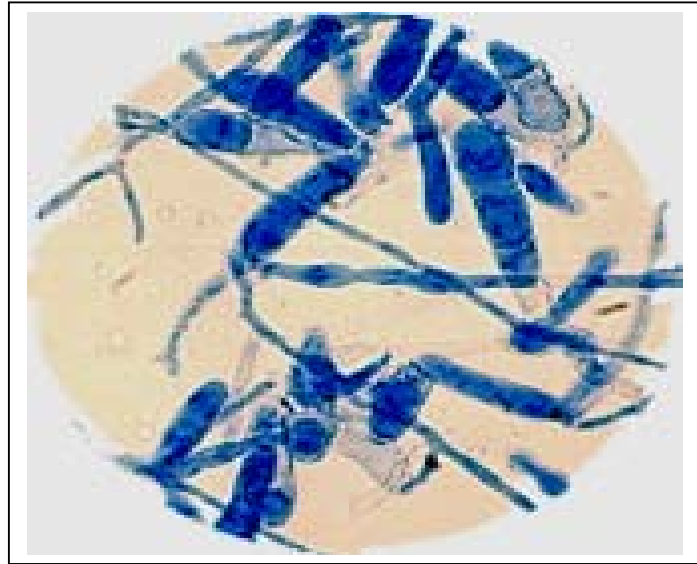
MICROSPORUM GYPSEUM



**EPIDERMOPHYTON FLOCCOSUM
(MACROSCOPIC)**



**EPIDERMOPHYTON FLOCCOSUM
(MICROSCOPIC)**



ANTIFUNGAL SUSCEPTIBILITY TESTING



FIGURE 9

CLINICAL PRESENTATION

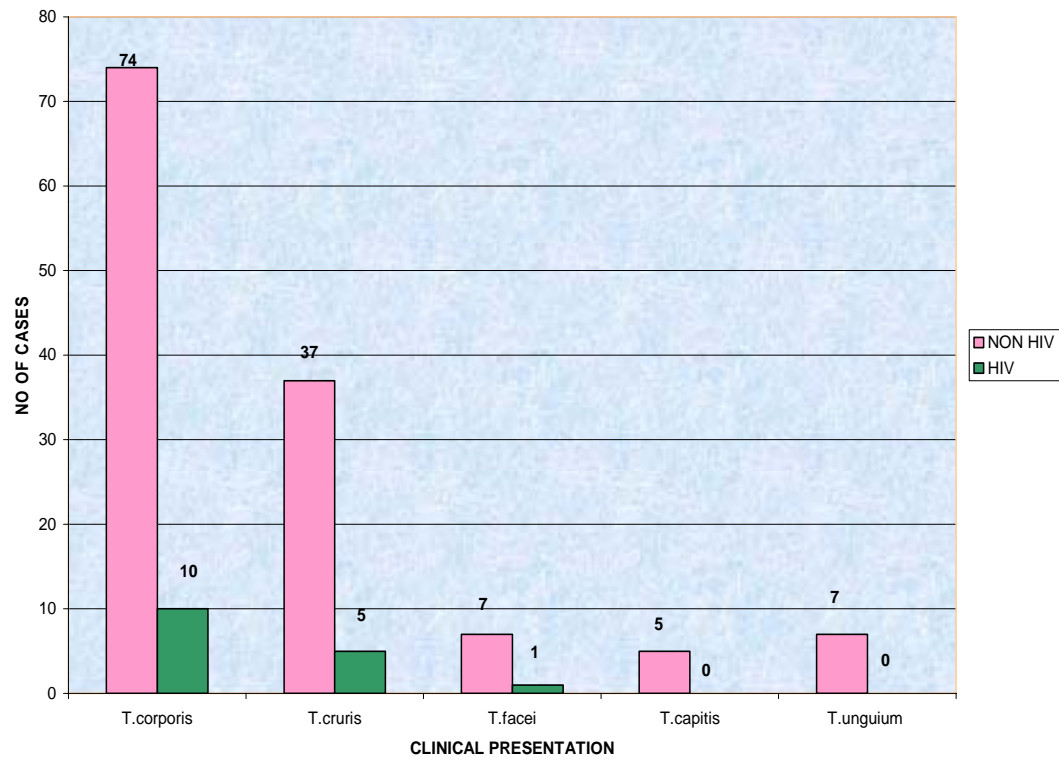
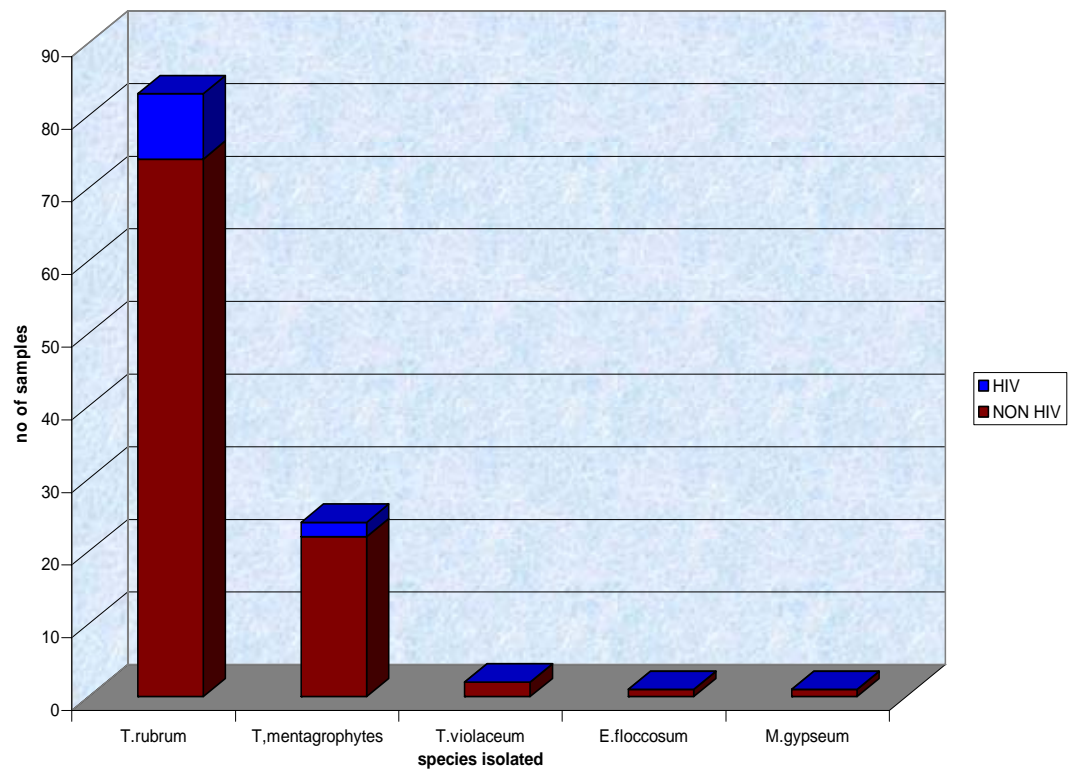


FIGURE 10
SPECIES ISOLATED



DERMATOPHYTE SPECIES ISOLATED

